

Observations on Osmotic Relationships in the Holothurian *Opheodesoma spectabilis*¹

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ABSTRACT: The means whereby *Opheodesoma spectabilis*, a holothurian confined to the quiet waters of Hawaii, can tolerate a dilution of 80% sea water were investigated. The animal is notable for the ratio of coelomic to tissue fluid, about 4:1.

Weight changes, osmometry, titrations, and sodium analyses on fluids before and after immersion of the animal in diluted sea water were some of the methods employed.

The animal was found to reduce its content of coelomic fluid when placed in dilute sea water for 24 hr. There is no rhythmic circulation or pumping of fluids. Water exchange and regulation of volume are by way of mouth and anus, as the body wall proved to be impermeable to water. Soluble salts of body fluids in both starved and feeding animals are below the concentrations found in sea water; but in feeding specimens, the coelomic fluid demonstrated osmotic activity approaching that of sea water.

It is suggested that tolerance to fresh water by the tissues is incidental, and derives from (1) the slow replacement of the comparatively large volume of coelomic fluid with environmental water, and (2) the habit of steady ingestion of variable quantities of organic material, leading to changing levels of digestive end-products in the coelomic fluid. Cells within the small volume of tissue fluid exchanging with the coelomic compartment can tolerate and have time to equilibrate with a wide range of concentrations, out of adaptive necessity. Volume regulation occurs in the gradual exchange between coelomic and environmental water to further advance osmotic equilibrium.

THE SEA CUCUMBER *Opheodesoma spectabilis* is a holothurian whose habitat appears to be confined to the relatively placid waters of Kaneohe Bay and Pearl Harbor of Oahu in the Hawaiian Islands (Edmondson, 1933; Fisher, 1907).

Although it is classed with the sea cucumbers, *Opheodesoma* differs from the more common types by having a thin, mucus-covered, smooth-muscle integument. This integument is usually stretched around an extraordinary amount of fluid contained in the coelomic cavity. This fluid, while it can be present in varying amounts from animal to animal and in the same animal from time to time, is most consistently found in a

ratio of about four parts to one part of tissue fluid. This comparatively large amount of mobile fluid is one of the salient features of the animal and apparently plays a significant role in its function.

In some other details of its physiology and anatomy, the animal is comparatively simple. It possesses a whorl of extensible-retractable tentacles representative of tube feet found in other echinoderms. These tentacles, usually 15 in number, surround the mouth and help the animal to ingest its food. The unconvoluted intestine, lacking the associated respiratory tree found in most other holothurians, is usually packed with a sand-algae mixture. The organic portion is digested and the end-products pass through the intestinal wall into the coelomic fluid. This fluid appears to act as the sole medium whereby nutrients are diffused to the peripherally located cell population.

¹ Contribution No. 237, Hawaii Institute of Marine Biology, University of Hawaii. Manuscript received August 31, 1964.

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Opheodesoma may be found in large numbers quietly feeding in the shallow waters over coral reefs. During heavy rains, when large amounts of fresh water run off from land to reefs located close to shore, the animal is unaffected by influxes of fresh water that decimate populations of other reef-dwelling invertebrates.

The primary objective of this work was to establish by what means these holothurians were able to function despite marked dilution of their sea water environment from time to time.

MATERIALS AND METHODS

Animals of an average length of 45 cm were collected from the Coconut Island reef, where at low tide they can be found as little as 10 cm below the surface of the water. The animals were kept in tanks supplied with a constant flow of aerated sea water. They were starved for 48 hr prior to experimentation. This provision was thought necessary because *Opheodesoma* is a detritus-algae feeder and the animals, upon first being taken from the water, differ from each other in the amount of sand and algae present in their intestines. After 48 hr the intestines were cleared in all animals.

Preliminary experiments indicated that the animals could survive in as low a dilution as 50% sea water for at least 24 hr. However, upon being returned to 100% sea water, death usually occurred within a matter of a few days. When placed in dilutions of 80% and 70% sea water for 24 hr, then removed to 100% sea water, animals survived up to three weeks. For further experimentation 80% sea water was chosen as representing the amount of dilution the animals might naturally encounter without being irreparably harmed.

Animals chosen for study were placed one each to a 5-liter glass tank containing a known ratio of sea water to distilled water. At least three animals were used in all dilution experiments, along with three control animals in 100% sea water. The tanks were aerated and topped with aluminum foil to prevent evaporation. Immersions were for 24-hr periods. The majority of experiments were carried out at room temperatures (23°–27° C).

Evaluations of changes due to water loss or uptake were made by comparing weights before and after the experimental immersion period. Osmotically active particles present in environmental water, coelomic fluids, and tissue fluids were determined by means of a Fiske cryoscopic osmometer. Sodium concentrations were measured by means of flame photometry. Titrations for chlorosity of sea water and body fluids as a measure of salt concentration followed Knudsen's procedure (U. S. Hydrographic Office, 1955).

RESULTS

Initial experiments were devoted to determining whether *Opheodesoma* could be categorized as either an osmoconformer or an osmoregulator.

Following a 48-hr starvation period, intact animals with coelomic fluid formed while in 100% sea water were weighed and placed in 80% sea water for 24 hr (Fig. 1).

It is evident from the data that the whole animal does not act as an osmometer. On the contrary, when in environmental water more dilute than body fluids, the animals lose rather than gain weight. Apparently some form of regulation does occur which runs counter to the simple osmotic relationship that would be expected in the experiment described. That is, if the integument of *Opheodesoma* is permeable to water as described for another holothurian by

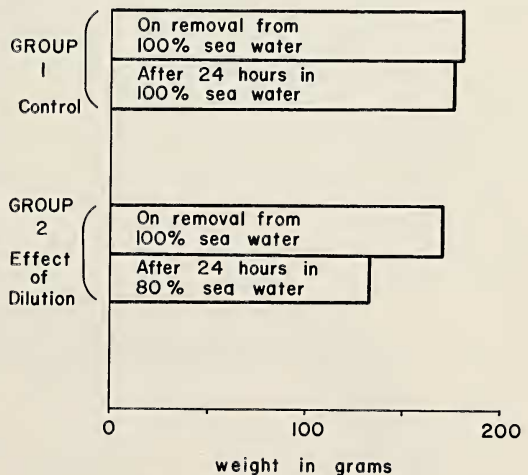


FIG. 1. Effect on animal weight after immersion in dilute sea water for 24 hr.

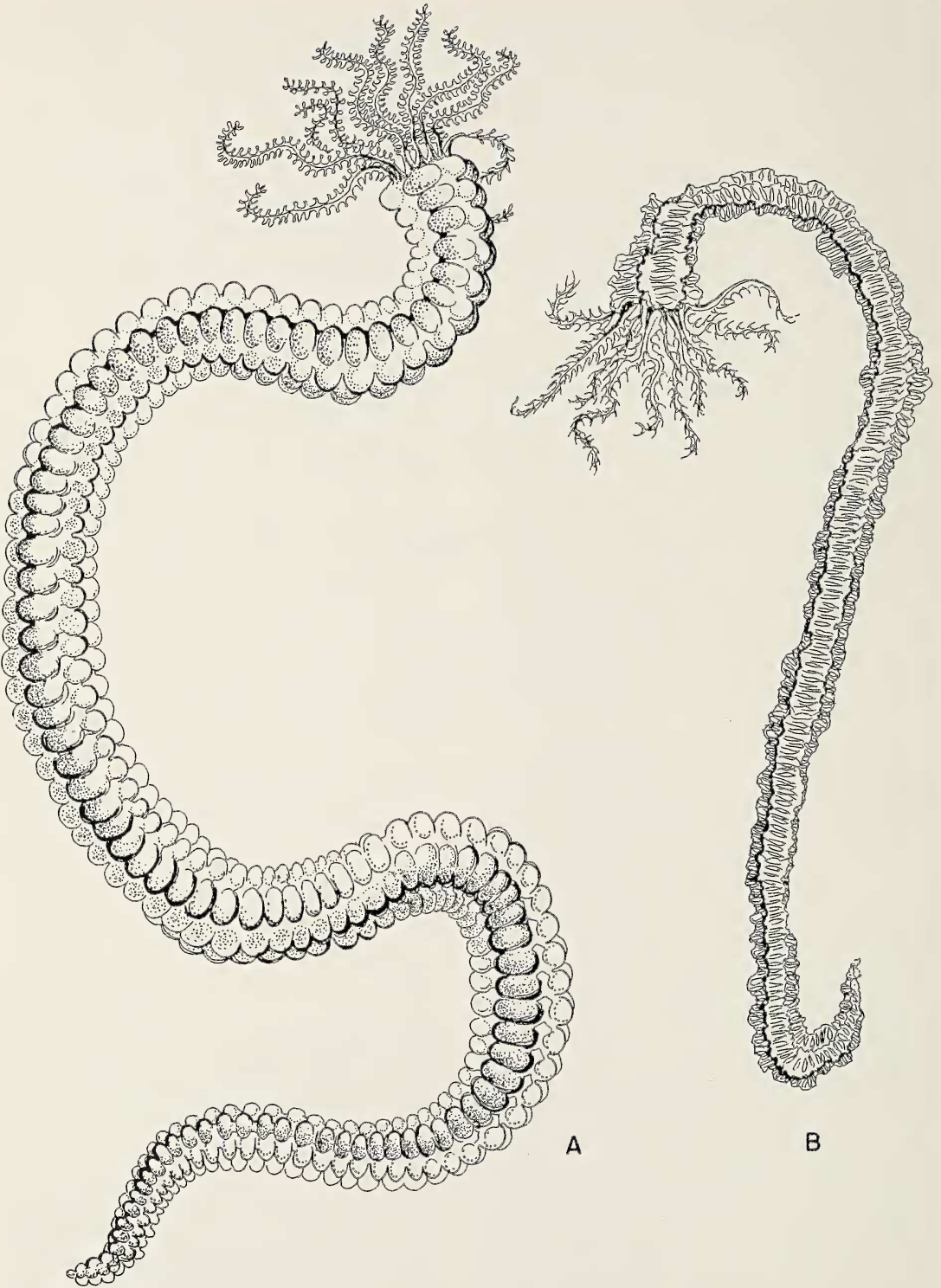


FIG. 2. *Opheodesoma spectabilis*. A, Fluid-filled state; B, drained state.

Koizumi (1935), and the body fluids are isotonic with the sea as has been found for most marine invertebrates (Prosser, 1961), then water should follow a gradient from outside to inside.

To determine which structures are involved in passing water might provide insight into the mechanisms of water volume regulation. The most direct approach was to find the water path used by drained animals in restoring their body fluids. Draining animals is a simple but important technique which has some bearing on conclusions to be drawn later; hence, it is described in some detail.

Opheodesoma under natural conditions is in a turgid state with the body wall distended through retention of a large volume of coelomic fluid (Fig. 2A). Out of water, the animal resembles an elongated balloon. If the animal is suspended by the head, the coelomic fluid flows to the tail region; the fragile integument stretches and usually ruptures, releasing the fluid. To induce animals to drain naturally through

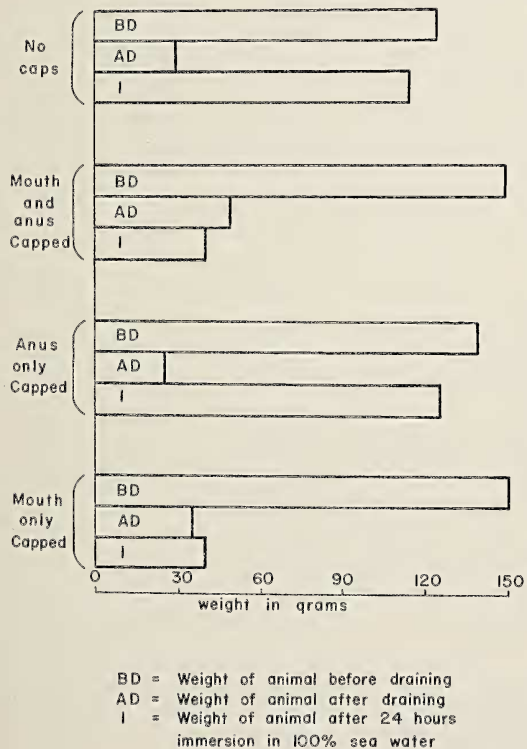


FIG. 3. Determination of functional site of water uptake in animals by use of plastic caps.

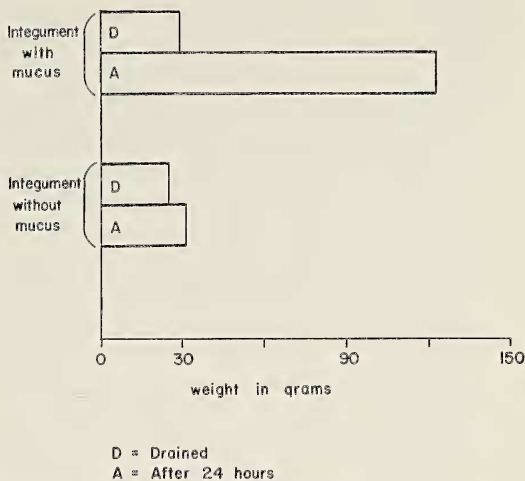


FIG. 4. The inability of animals with mucus-free integuments to restore coelomic fluid after 24 hr in 100% sea water.

the anus, they were supported in a horizontal position so that the body wall would not rupture and a 20-gauge needle was inserted carefully into the anus. Gentle manipulation with the needle usually provided the stimulation necessary to release the sphincter muscle. The fluid then would drain, leaving the animal considerably reduced in size (Fig. 2B). Upon being returned to 100% sea water, *Opheodesoma* would immediately start restoring coelomic fluid, and by the end of 24 hr it would regain an amount close to that of its original volume. If rupture of the body wall did occur at any time in the course of an experiment, the animal was discarded.

In the experiments animals were first weighed upon being removed from storage tanks. They were drained, reweighed, fitted with plastic caps in the various combinations indicated in Figure 3, and then returned to sea water for 24 hr. Water intake evidently occurs only through the mouth, with the integument acting as a water-tight barrier.

The importance of the mucus coating of the integument in making it impermeable to water was established by gently wiping off the mucus with a towel, draining the coelomic fluid from the animals, and returning them to sea water for 24 hr. As is evident from Figure 4, animals so treated usually lose the ability to regain fluid.

Volume regulation and the normal route of water exchange with the environment is via mouth and anus. But is the exchange intermittent or continuous? To test these alternatives, 1 ml of 0.1% safranin dye was injected through the body wall directly into the coelomic fluid. The animals were then washed and returned to the clear water of their natural environment in algal beds on the reef, so that feeding would not be interrupted if feeding entailed water circulation. The colored coelomic fluid could easily be seen through the semitransparent skin. During 2 hr of close observation no apparent coloring of water around the animals was detected. Removing them from the reef and draining them showed that the coelomic fluid still contained dye. Animals with coelomic fluid intact when placed in tanks containing dye-colored sea water,

showed no dye uptake after 4 hr of immersion. However, the animals were not feeding.

If water is not circulating by the mouth-anus route in a regular fashion, fluids must be "turned over" by the animals very slowly. Apparently there is no physiological demand for a constant flow of water as a carrier for oxygen or food. Oxygen could diffuse directly into and across the integument where most of the cell population is concentrated. This conclusion is supported indirectly by the fact that these particular holothurians lack the respiratory trees found in thick-walled species (Hyman, 1955).

While no direct study was made on nutritional habits, casual observation indicated an almost solid stream of sand mixed with algae being waved into the mouth by rhythmic tentacle activity. The intestine may be filled solid with

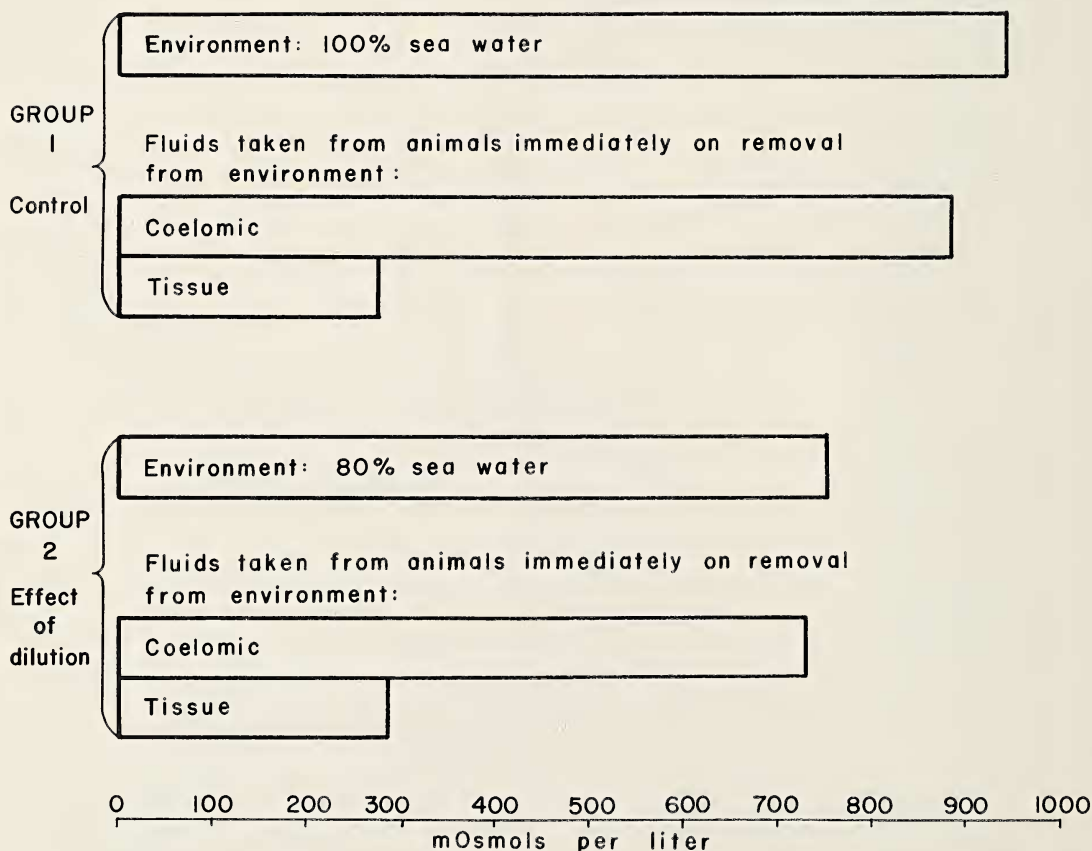


FIG. 5. The osmotically active concentrations of coelomic and tissue fluids of animals immersed in 100% and 80% sea water for 24 hr.

this mixture, while the animal is continually extruding pellets free of any vegetable matter. However, animals can vary markedly in their nutritional activity, even when lying side by side.

Evaluation was made of osmotically active substances of fluids from coelomic and tissue compartments before and after osmotic stress. For coelomic fluid determinations, draining was induced and samples were taken. Animals were either returned to 100% sea water, as in the case of the controls, or were immersed in dilute sea water. All animals were kept in tanks for 24 hr; then draining was repeated and samples were taken. Tissue fluid was obtained by drying drained animals thoroughly in toweling and then macerating them in a Waring blender. The resulting homogenate was pressed through a fine fibreglass mesh and finally filtered.

Figure 5 shows that the mOsmol values for coelomic fluids are slightly lower than for en-

vironmental water. The low concentration of tissue fluid is surprising, if it is assumed that a concentration equilibrium usually exists between body and environmental fluids in marine invertebrates. The fact that tissue fluid values are nearly the same whether or not sea water was diluted in the environment is also difficult to explain. It was hypothesized that draining the animals in the manner described might be instrumental in removing salt from the tissues. To test this possibility, coelomic fluid samples were obtained in two different ways from the same animal. One method was by draining as usual, the other was by aspiration of a small amount of fluid directly through the body wall by using a needle and syringe.

The concentrations in samples so obtained are compared in Figure 6, both in mOsmols of unspecified substances and in μ Mols of sodium. The significantly higher values of the drained

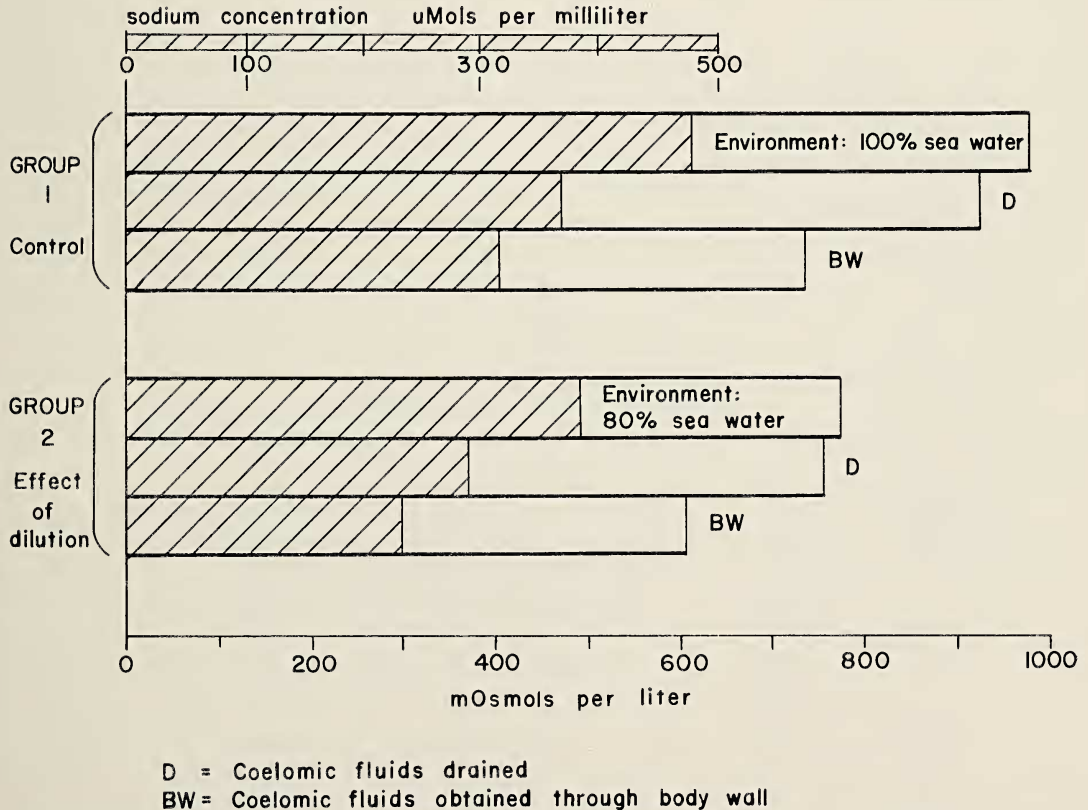


FIG. 6. Comparison of concentration of coelomic fluids obtained in two different ways. Immersion in 100% and 80% sea water for 24 hr.

TABLE 1

WEIGHT CHANGES IN ANIMALS DRAINED OF COELOMIC FLUID BEFORE AND AFTER BEING IMMERSSED IN 100 AND 80% SEA WATER FOR 24 HR

ANIMAL NO.	INITIAL DRAINED WT AFTER REMOVAL FROM 100% SEA WATER (grams)	DRAINED WT AFTER 24 HR IN 100% SEA WATER (grams)	DRAINED WT AFTER 24 HR IN 80% SEA WATER (grams)
1	37.4	35.2	
2	31.4	29.3	
3	39.5	37.2	
4	31.0	29.2	
5	36.4		40.1
6	37.0		39.8
7	33.8		37.0
8	29.1		33.7
9	28.2		31.3

over the *in situ* coelomic samples point to a salt flow from the tissue when animals are drained. Analysis for sodium alone was done as an index to the possibility of differential ion regulation occurring between cells and coelomic fluid as an aid to osmotic balance (Robertson, 1957). The fact that sodium concentrations are propor-

tional to mOsmol values throughout indicates that ion regulation is of little importance in achieving osmotic equilibrium.

Attention was given to the wet weight of tissues as a possible quantitative index of volume regulation during stress. The data in Table 1 reveal a consistent pattern of tissues gaining

TABLE 2

WEIGHT MEASUREMENTS AND CALCULATIONS INDICATING RATIO OF SIZES OF COELOMIC TO TISSUE COMPARTMENTS*

ANIMAL NO.	A WT WITH COELOMIC FLUID (grams)	B WT AFTER DRAINING (grams)	C WT AFTER DESICCATION (grams)	A-B COELOMIC COMPARTMENT (grams)	B-C TISSUE COMPARTMENT (grams)	RATIO OF COMPARTMENTS (COELOMIC: TISSUE)
1	165.2	39.9	2.9	125.3	37.0	3.4:1
2	134.3	24.0	2.4	114.3	21.6	5.1:1
3	156.3	29.5	2.7	126.8	26.9	4.7:1
4	124.5	33.2	2.5	91.3	30.7	3.0:1
Average	145.7	31.6	2.6	113.1	29.5	3.9:1

* One of two experiments.

water after 24 hr in dilute sea water, in contrast to the controls which lose water over the same time interval. Initially, if salt is washed out of the tissues by draining, the wet tissue weights measured after immersion in different sea water concentrations are indicative of how osmotic equilibrium may be regained. In the case of the controls, the 100% salt water is more concentrated than the washed-out tissue, and water moves out from cells. However, when the restored coelomic fluid is more dilute, the water moves from the coelomic compartment to the tissue cells.

This indicates that changes in coelomic fluid concentration can exert a sizable effect on what goes on in the tissues. This may be better appreciated by inspecting the data shown in Table 2. The amount of mobile fluid in the coelomic cavity constitutes an average of 77% of the total body weight, while the coelomic compartment itself is nearly 4 times that of the tissues. In

addition, the tissue is so arranged that most of it is stretched into a thin, membrane-like structure encompassing the coelomic fluid, so that a quick, thorough exchange between tissues and coelomic compartments is feasible.

The comparatively low concentrations found for tissue fluid might result from the imposed 48-hr starvation period. This possibility was evaluated by taking samples directly from animals feeding on the reef and comparing the concentrations with those from starved animals, by both osmometry and chlorosity titrations. Thus, osmotic activity due to salts can be distinguished from that due to organic molecules.

The data in Table 3 show that while feeding did not increase the tissue fluid mOsmol concentration, it certainly raised the mOsmol concentration of *in situ* coelomic fluid. Coelomic osmotic activity in feeding animals now approaches that of sea water but, as the chlorosity values for both feeding and starved animals are

TABLE 3

COMPARISON OF CONCENTRATIONS OF COELOMIC AND TISSUE FLUIDS IN FEEDING AND STARVED ANIMALS*

ANIMAL NO.	COELOMIC FLUID				TISSUE FLUID mOsmols/L
	DRAINED		THRU BODY WALL		
	Chlorosity†	mOsmols/L	Chlorosity	mOsmols/L	
Feeding					
1	18.5	868	15.7	936	248
2	17.2	760	14.0	808	274
3	17.9	815	14.8	876	257
Average	17.8	811	14.8	873	260
Starved					
4	18.8	848	13.4	587	234
5	18.7	868	13.8	664	268
6	18.0	825	14.4	697	270
Average	18.5	847	13.8	649	257

* One of two experiments.

† Chlorosity = grams of chloride per liter. Chlorosity values for tissue fluids could not be obtained due to insufficient volumes.

nearly the same, the difference can be attributed to the end products of digestion.

DISCUSSION

The easy tolerance of *Opheodesoma* to dilution of its normal sea water environment (up to 70% with no apparent harm) is puzzling. The animal has no particular structures devoted to osmoregulation; yet, the impermeability of the skin and the lack of rhythmic water circulation remove it from the category of osmoconformity. There appears to be no direct adaptive significance in tolerance extending *Opheodesoma's* environmental range. The animal is usually confined to quiet reef living, and while the ability to resist dilution may have some survival value in times of heavy rainfall for animals in shallow water, they have been found at depths up to 40 ft, presumably never exposed to fresh water.

Opheodesoma is normally a continuous ingester. This may call for a compensatory reduction in the soluble salt content of the body fluids when the organic content is high. Then the influx of digestive end-products into the large coelomic compartment raise its osmotic pressure to nearly that of sea water. Lange (1963), in his study on *Mytilus*, postulated a similar role for organic solutes acting as an osmotic buffer. Krogh (1939) cited Fredericq's finding that the tissues of certain marine invertebrates, while in osmotic equilibrium with the surrounding water, might show considerable deficit in soluble salts. This deficit is made up by organic molecules of low molecular weight.

Despite irregular water exchange with the environment and variable organic molecule levels, the volume and salt level of the coelomic fluid of *Opheodesoma* in its natural environment appear to have a certain stability. The values of the fluid samples taken through the body wall are probably more representative of actual concentrations due to the relatively undisturbing nature of the method. The inference is made here that the concentration differences between drained and *in situ* coelomic fluids must be accounted for in the tissues (Fig. 5). Consequently, the values reported for tissue fluids are lower than they actually would be *in vivo*, as measurement is possible only on drained animals. These

measurements really represent, then, a residual concentration after draining has removed some of the soluble substances which normally help keep osmotic equilibrium between tissue and coelomic fluids.

It is suggested that tolerance to fresh water by the tissues is incidental and derives from (1) the slow "turnover" of the comparatively large volume of coelomic fluid with environmental water, and (2) the habit of steady ingestion of variable quantities of organic material by the animal, leading to oscillating levels of digestive end-products in the coelomic fluid. Cells within the small volume of tissue fluid exchanging with the coelomic compartment have time to equilibrate with a range of concentrations, and can tolerate them out of adaptive necessity.

Submitting *Opheodesoma* to dilutions of sea water of the order they might naturally encounter in terms of fresh water runoff creates no critical problem, particularly when ingestion is slowed or stopped. The resulting reduction in the level of organic molecules in coelomic fluid would lower its osmotic activity. New osmotic equilibrium would be established between tissue and coelomic fluids. Then, as gradual exchange between coelomic and environmental water occurs via the mouth-anus route, reduction of coelomic volume follows as a necessary condition for complete osmotic equilibrium.

ACKNOWLEDGMENTS

This investigation was performed while the author was on sabbatical leave from San Jose State College. He wishes to express his gratitude for the assistance and advice given by the Hawaii Marine Laboratory Staff.

Use of the Fiske osmometer was made available through the kindness of Dr. Ira Lichten and Miss Hester Kobayashi of the Department of Nutrition, University of Hawaii.

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